STRUCTURAL AND BIOMECHANICAL ALTERATIONS ASSOCIATED WITH PLATELET-DRIVEN CLOT CONTRACTION

Oleg V. Kim[a,b,c], Rustem I. Litvinov[a], Mark S. Alber[b,c], and John W. Weisel[a]

[a]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [b]Department of Mathematics, University of California Riverside, Riverside, CA
[c]Department of Applied and Computational Mathematics and Statistics, University of Notre Dame, Notre Dame, IN

Abstract

The volume shrinkage of blood clots named clot contraction (retraction) that determines the final size and structure of a mature clot is an essential part of blood clotting. Platelet-driven clot contraction is important for hemostasis and wound healing as well as for restoring the blood flow past otherwise obstructive thrombi within a vessel. While it has been demonstrated that platelets and fibrin are necessary for contraction of clots, much less is known about how individual platelets or small platelet aggregates exert contractile force on individual fibrin fibers and how this tension causes collapse of the entire filamentous network and reduction of clot volume. The studies described so far define the components necessary for clot contraction, but the physical mechanism is still unknown. ***In other words, what is the physical action of platelets that causes contraction of the clot and what structural alterations in fibrin occur during cell-based clot contraction?*** To gain insight into the structural reorganization of the extracellular matrix underlying platelet-driven clot contraction biomechanics, we used high-resolution confocal microscopy and rheometry to perform concurrent 3D dynamic structural and mechanical measurements of the platelet-fibrin meshwork over the course of clot contraction. We paid special attention to the elementary steps of clot contraction in the real time scale by visualizing single contracting platelets bound to an individual fibrin fiber and their effects on remodeling of the entire fibrin network powered by multiple contracting platelets.

Materials and Methods

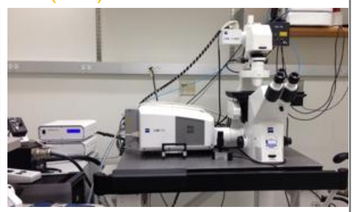
Alexa-Fluor 594-labeled human fibrinogen

calcein green, AM (Molecular Probes)

+ $CaCl_2$ (40 μM final), thrombin (0.75-1 U/ml final)

Platelet-Rich Plasma (PRP)

AR-G2 Rheometer
(TA Instruments, New Castle, DE)



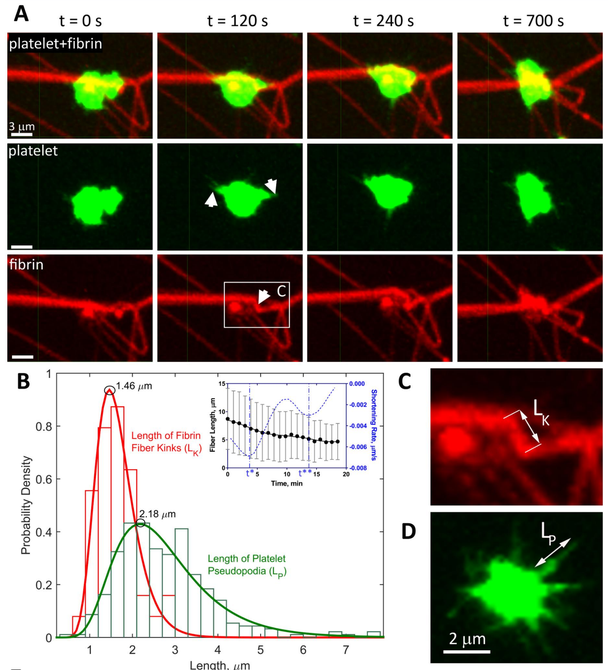
Zeiss LSM 710

Clot mechanical properties (G', G'')

Structural characteristics of the clot

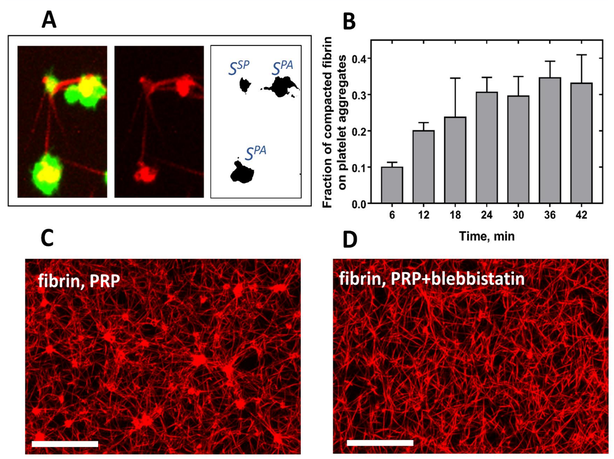
U01HL116330

Results

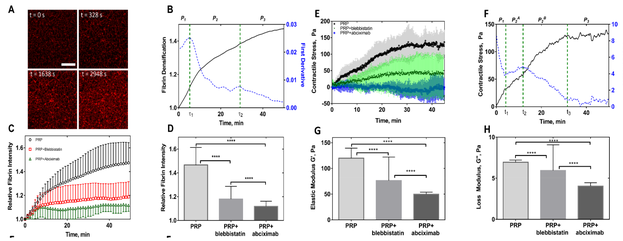


Time-lapse images of contracting platelets that cause bending, kinking and local accumulation of a single fibrin fiber. (**A) Top row**: A platelet or a small platelet aggregate (green) attaches to a fiber (red) and spreads filopodia along the fiber axis that contract, inducing a fiber kink and pulling the fiber, compacting it into a dense fibrin knot or coil. (**A**) **Middle row**: Platelet transformations, including attachment of filopodia to a fiber, spreading and contraction (corresponding to A, **Top row**). (**A) Bottom row**: Platelet-induced structural changes in a fibrin fiber. The inset shows formation of a kink. (**B)** Length distributions of the fiber kinks, L_K (shown in **C**) and platelet filopodia, L_P (shown in **D**); t*, t** are the microscopic phase transition times separating different regimes of filopodia shortening. (**E**) Serial images of a contracting platelet reveal reorganization and compaction of fibrin fibers surrounding the cell

Results



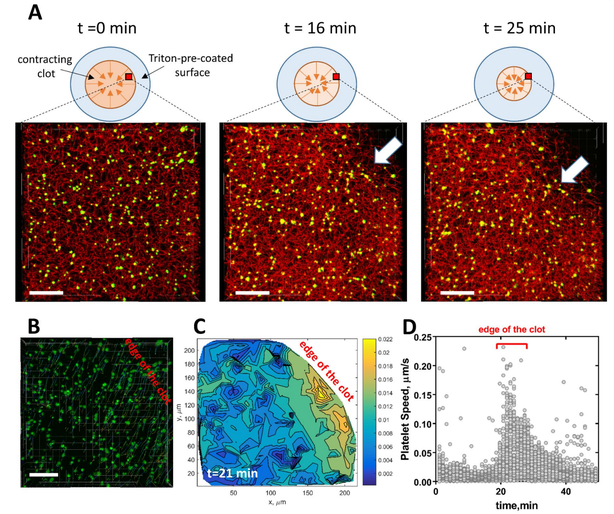
Platelet-induced fibrin compaction. (**A, left):** a confocal image of platelets and fibrin; (**A, center):** fibrin fibers and patches (compacted fibrin); (**A, right):** fibrin co-localized with platelets. Compaction of fibrin by a single cell (S^{SP}) and by two platelet aggregates (S^{PA}) is shown. (**B)** Relative portion of fibrin matter compacted by platelet aggregates, M±SEM, N>200. (**C, D)** Confocal images of a fibrin network in a PRP-clot formed and allowed to contract in the absence (**C**) and presence (**D**) of blebbistatin. Magnification bar = 30 μm.



Structural contraction kinetics of PRP-clots. A: Serial confocal images showing time-dependent densification of the fibrin network. **B:** Fibrin densification characterized by three phases determined by the local extremes of the first derivative. **C:** Fibrin densification as a function of time in the absence and presence of blebbistatin and abciximab. **D:** Fibrin fluorescence intensity (fibrin density) at the end of contraction in the absence and presence of blebbistatin and abciximab.

Mechanical contraction kinetics of PRP-clots. **E:** Dynamic contractile stress generated by the platelet-fibrin meshwork in the absence and presence of abciximab and blebbistatin. **F:** The kinetic curve shown in **E** has four phases defined as in **B**. (**G,H):** The storage and loss moduli of fully contracted PRP clots (50 min) in the absence and presence of blebbistatin and abciximab.

Results



Non-uniform deformation of platelet-fibrin meshwork during clot contraction. Time-lapse z-stack confocal imaging of the platelet-fibrin meshwork revealed drastic differences in the speed of translocating platelets at the edge of the contracting clot moving inwards and inside the clot **(A, B)**. Spatial (**C**) and temporal (**D**) resolution of platelets movement speed. Spatial anisotropy of contracting clots is due to the faster moving edges and less mobile clot's interior domains.

Conclusions

- **Our study provides quantitative structural details of clot contraction.**
- **Activated platelets bend and shorten individual fibrin fibers via their filopodia that undergo sequential extension and retraction, as if pulling hand-over-hand.**
- **Platelets induce compaction of fibrin fibers into platelet-attached agglomerates.**
- **Platelet contraction causes secondary fibrin-mediated platelet aggregation**
- **Contracting platelets actively remodel the fibrin network by increasing its density followed by enhancement of clot stiffness.**
- **Kinetic analysis revealed a multiphasic behavior at the macro- and microscales with at least three distinct phases that differ in duration and rate constants.**

References

1. Kim, O.V., Litvinov, R.I., Alber, M.S., and Weisel, J.W. 2017. Quantitative structural mechanobiology of platelet-driven clot contraction. Nat. Com. 8(1) 1274.
2. Höök, P., Litvinov, R.I., Kim, O.V., Xu, S., Xu, Z., Bennett, J.S., Alber, M.S. and Weisel, J.W., 2017. Strong binding of platelet integrin αIIbβ3 to fibrin clots: potential target to destabilize thrombi. *Scientific reports*, *7*(1), p.13001.
3. Le Minh, G., Peshkova, A.D., Andrianova, I.A., Sibgatullin, T.B., Maksudova, A.N., Weisel, J.W. and Litvinov, R.I., 2018. Impaired contraction of blood clots as a novel prothrombotic mechanism in systemic lupus erythematosus. *Clinical Science*, *132*(2), pp.243-254.
4. Tutwiler, V., Wang, H., Litvinov, R.I., Weisel, J.W. and Shenoy, V.B., 2017. Interplay of Platelet Contractility and Elasticity of Fibrin/Erythrocytes in Blood Clot Retraction. *Biophysical Journal*, *112*(4), pp.714-723.